14

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PC-2006663	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE00/01491	International filing date *(day/month/year)* 13.07.2000	Priority date *(day/month/year)* 13.07.1999
International Patent Classification (IPC) or national classification and IPC7 A61K 38/20, A61K 38/22, A61P 3/04, A61P 3/06		
Applicant SALTECH I GÖTEBORG AB et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
31.01.2001	08.11.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Yvonne Siösteen/EÖ Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE00/01491

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement) under article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PR[redacted]MINARY EXAMINATION REPORT

[redacted]rnational application No.
PCT/SE00/01491

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 17-30

because:

☒ the said international application, or the said claims Nos. 17-30
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1(iv): Methods for treatment of the human or animal body by therapy.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims. or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE00/01491

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	4-8, 11-16	YES
	Claims	1-3, 9-10	NO
Inventive step (IS)	Claims		YES
	Claims	1-16	NO
Industrial applicability (IA)	Claims	1-16	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The present application pertains to the use of a substance that upon administration to a patient will lead to an increased level of interleukin-6 (IL-6) receptor agonist for the production of a medical product for treatment of obesity and/or obesity associated disorders (second medical indication).

The wordings "a substance" and "interleukin-6 (IL-6) receptor agonist" in claims 1-2 and the wording "a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist" in claim 14 relate to a very large number of possible substances. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the substances claimed. The international search has therefore mainly been focused on the examples given in the application i.e. IL-6 and leptin. Consequently, this international preliminary examination report is based on what has been covered by the search.

The wording "obesity associated disorders" in claims 1 and 4 relates to a large number of possible disorders. However, the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very small proportion of the disorders claimed. Specifically, support is found for obesity as such, for disturbances in serum triglyceride levels and in circulating levels of leptin. Claim 1, as it is formulated, does not fulfill the requirements of Art 5 and 6 PCT.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

D1: File WPI, Derwent accession no. 1993-269760, TORAY IND INC: "Hypolipidaemic drug for efficient cholestrerol redn. in blood and compatibility - comprises interleuk in 6 prepd. by human cell culture excluding contamination and antibody prodn. On admin. Into body, avoiding enzymatic reaction redn.";
& JP,A,5186367, 19930727, DW199334

D2: Am. J. Physiol., Volume 275, 1998, Davide Agnello et al, "Leptin causes body weight loss in the absence of in vivo activities typical for cytokines of the IL-6 family" page 913 - page 919

D1 relates to a hypolipidemic drug comprising interleukin-6. The drug is useful for the reduction of cholesterol in blood. Cardiovascular diseases, which are generally recognised as being obesity associated disorders, are associated with, amongst other factors, high blood lipid levels. Blood lipids include cholesterol and triglycerides. Therefore, claims 1-3 and 9 are considered to lack novelty in relation to D1. Claim 10 relates to the metabolic syndrome, which includes disturbances of blood lipids. Therefore, claim 10 is considered to lack novelty in relation to D1. Further, that the obesity or obesity associated disorders may be caused by a pathological disturbance of fat metabolism, is considered to be an obvious possibility to a person skilled in the art. It is also considered that a person skilled in the art would try to use IL-6 in the treatment of other risk conditions associated with obesity, such as high levels of serum triglycerides and diabetes type II. Consequently, claims 4 and 8-11 are considered to lack inventive step in relation to D1. It is further considered that claims 5-7 and 12-13 constitute obvious embodiments of the invention that seem to be obvious to a person skilled in the art. Accordingly, claims 5-7 and 12-13 are considered to lack inventive step.

D2 reveals that leptin causes body weight loss. Furthermore, IL-6 has been observed to cause body weight loss and/or food intake reduction (see page R916, 1st column). It is also shown in D2 that leptin potentiates the induction of IL-6. Therefore, it is considered to be obvious to a person skilled in the art to use IL-6 alone or in combination with leptin in the treatment of obesity and obesity associated disorders. Consequently, claims 1-16 are considered to lack inventive step in relation to D2.

VIII. *Certain observations on the international application*

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The wordings "a substance" and "interleukin-6 (IL-6) receptor agonist" in claims 1-2 and the wording "a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist" in claim 14 relate to a very large number of possible substances. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the substances claimed. The international search has therefore mainly been focused on the examples given in the application i.e. IL-6 and leptin. Consequently, this international preliminary examination report is based on what has been covered by the search.

The wording "obesity associated disorders" in claims 1 and 4 relates to a large number of possible disorders. However, the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very small proportion of the disorders claimed. Specifically, support is found for obesity as such, for disturbances in serum triglyceride levels and in circulating levels of leptin. Claim 1, as it is formulated, does not fulfill the requirements of Art 5 and 6 PCT.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/03725 A1

(54) Title: USE OF INTERLEUKIN-6 IN TREATMENT OF OBESITY AND/OR OBESITY ASSOCIATED DISORDERS

(57) Abstract: Use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist, preferably IL-6, for the production of a medicinal product for treatment of obesity and/or obesity associated disorders is disclosed. Also a method for treatment of obesity and/or obesity associated disorders wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 receptor agonist is disclosed.

USE OF INTERLEUKIN-6 IN TREATMENT OF OBESITY AND/OR OBESITY ASSOCIATED DISORDERS

Technical field of the invention

The present invention relates to a new medicinal product and a new method for treatment of pathological disturbances of regulation of body fat tissue mass and/or obesity associated disorders.

Background art

Understanding obesity

Obesity is a large problem in the Western world since both severe and moderate obesity is associated with increased health risks. Obesity is associated with diseases such as diabetes, hypertension and heart disease, whose incidence increases with body-mass index (BMI, body mass in kg/square of height in meters). A study based on information on 18-year-old Swedish military conscripts show a 1.4-fold increase in prevalence of overweight (BMI >25) and a 1.7-fold increase in obesity (BMI >30) from the year 1971 to 1993 (Rasmussen F, Johansson M and Hansen HO, 1999).

Generally, obesity is due to energy intake that exceeds energy expenditure. This can be caused by overeating, i.e. higher food intake than necessary for maintenance of body mass. In addition, low mobility and low metabolic rate may predispose for obesity (see Flier, J. S. and Foster D. W. (1998) Eating disorders: obesity, anorexia nervosa, and bulimia nervosa. In: Williams Textbook of Endocrinology, 9th Ed, Saunders Co.).

However, the general opinion that obesity is largely the result of a lack of willpower is unsatisfactory. Intense research efforts are therefore made to reveal the genetic and environmental factors of importance for development of obesity (Friedman JM and Halaas JL, 1998).

Obesity in humans and mice

Animal models can be used for investigation of which genes that are causing development of obesity. Of particular importance is the information that can be gained from mouse strains that develop obesity because of gene knockouts. These mouse strains can provide evidence that a certain gene product is of crucial importance for regulation of body fat. This in turn may facilitate the development of new treatment paradigms. There are indications that there are gender differences regarding the genetic ethiology of obesity (see e.g. Costet, P. et al. (1998) Peroxisome Proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. J. Biochem. Chem. 273,29577-29585).

Obesity and blood fats in relation to cardiovascular disease

It is recognized that obesity, especially visceral obesity, and deranged lipid-lipoprotein profile, including hypertriglyceridemia and hypercholesteolemia are associated with larger risk of cardiovascular disease (Lamarche B, et al. (1998), Visceral obesity and the risk of ischemic heart disease: insights from the Quebec cardiovascular study. Growth hormone and IGF research 8, (suppl. B) 1-8.). So far, a lot of the research on the ethiology of this syndrome has dealt with neuroendocrine, i.e. hypothalamohypophyseal, and endocrine disturbances, focusing on the effects of the hypothalamus-pituitary-adrenal (HPA) axis regulating glucocorticoid, sex steroids and growth hormone (see e.g. Björntorp, P. (1996) The regulation of adipose tissue distribution in humans, Int. J. Obesity 20, 291-301.)

Leptin and obesity

Following the cloning of leptin 6 years ago (see Zhang et al. (1994), Positional cloning of the mouse *ob*

(obesity) gene and its human homologue. Nature 372, 425-432), there were great hopes that this would mean new possibilities to treat obesity and overeating. However, later it was found that obesity in humans very seldom is due to leptin deficiency, but rather is associated with increased leptin levels. Moreover, it has been shown that both mice and humans often are resistant to the anti-obesity effect of leptin (see e.g. Flier, J. S. (1998), What's in a name? In search of leptin's physiological role, J Clin. Endocr. Metab 83, 1407-1413, and references therein).

The 16 kDa protein leptin is almost only produced in white adipocytes from which leptin is then released to circulation. Leptin production by fat and plasma leptin levels is highly correlated with adipose tissue mass (Flier JS, 1997). Leptin acts through specific receptors in the hypothalamus to create a feedback loop for body weight regulation. Therefore, the pathophysiology of obesity was assumed to be partly endocrine. Leptin does not rise significantly after a meal and does not result in the termination of a meal. Instead leptin appears largely to exert long-term effects on food consumption and energy expenditure (Flier JS, 1998; Friedman JM and Halaas JL, 1998).

Leptin as a starvation signal

Obese (*ob*) mice which lack leptin show many of the abnormalities seen in starved animals, including hyperphagia, decreased body temperature, decreased energy expenditure, decreased immune function, and infertility. Leptin replacement corrects all of these abnormalities implying that *ob* mice live in a state of "perceived starvation" due to lack of leptin and that the biological response in the presence of food leads to obesity. These observations led to speculation that leptin's main physiological role is to signal nutritional status during

periods of food deprivation (Flier JS, 1998; Friedman JM and Halaas JL, 1998).

The leptin receptors

The leptin receptor (Ob-R) is normally expressed at high levels in hypothalamic neurons and in other cell types, including T cells and vascular endothelial cells. *In situ* hybridisation was used to identify the hypothalamic arcuate nucleus, and also dorsomedial hypothalamic nucleus (DMH), paraventricular nucleus (PVN), ventromedial hypothalamic nucleus (VMH) and lateral hypothalamic nucleus (LH) as principal sites of Ob-R expression in the central nervous system. Each of these nuclei, such as the arcuate nucleus, express one or more neuropeptides and neurotransmitters such as neuropeptide Y (NPY) and melanocyte-stimulating hormone alpha (-MSH), that regulate food intake and/or body weight, probably by actions downstream of leptin (Friedman JM and Halaas JL, 1998; Flier JS and Maratos-Flier E, 1998).

Leptin and human obesity

The role of leptin in the pathogenesis of obesity may be inferred by measurement of plasma leptin. An increase in plasma leptin suggests that obesity is the result of resistance to leptin. A low or normal plasma concentration of leptin suggests that obesity is due to decreased production of leptin. This interpretation is similar to that used in studies of insulin and the pathogenesis of type I and type II diabetes. As is the case with insulin and its receptor in diabetes, mutations of leptin and its receptor are rare in human obesity, but most obese individuals still have higher levels of leptin than do non-obese individuals, an indication of leptin resistance that might be receptor-independent (Flier JS, 1997).

Many genes involved in development of obesity have recently been found and most of them seem to act down-

stream of leptin at the hypothalamic level. Other genes that are involved in development of obesity encode neuropeptides, e.g. leukocyte adhesion receptors, which are important cell-cell adhesion molecules in the inflammatory and immune systems (Dong ZM *et al.*, 1997), and neurocytokines like ciliary neurotrophic factor, whose receptor subunits share sequence similarity with the leptin receptor (Gloaguen I *et al.*., 1997). The identification of anti-obesity mechanisms that act independently or together with the leptin system may help to develop strategies for the treatment of obesity associated with leptin resistance.

Leptin has immuno-regulatory activity

Exogenous leptin up-regulates both phagocytosis and the macrophage production of proinflammatory cytokines such as tumor necrosis factor (TNF-) and interleukin-6 (Loffreda S *et al.*, 1998). It has been suggested that the up-regulation of inflammatory immune responses by leptin may contribute to several of the major complications of obesity such as increased incidence of infection, diabetes and cardiovascular disease (Loffreda S *et al.*, 1998; McCarty MF, 1999). This hypothesis is attractive since it would implicate a common pathogenic mechanism (lack of leptin action) for both obesity and some of its major complications. However, an alternative possibility is that regulatory mechanisms usually connected to e.g. immune functions also are of importance for the regulation of body fat.

Interleukin-6

The cytokines act as hormonal regulators of the immune system and in the body's reactions during trauma and inflammation. The cytokine interleukin-6 (IL-6) is known to be important in the development of B-lymphocytes and in the change of plasma protein production of the liver during trauma and inflammation, the so-called acute phase

response. In line with this, IL-6 levels are markedly increased during acute phase response. It has been shown that IL-6-type cytokine receptors share functional specificity with the long form of the leptin receptor (Baumann H *et al.*, 1996). The role of the cytokines including IL-6 in healthy animals and humans is not well known and they are suggested to have little effect, partly because circulating levels often are low in the absence of illness (Hirano T, 1998).

Structures of interleukin-6 and its receptor

Interleukin-6 (IL-6) exerts its biological effects through the ligand-specific IL-6 receptor, which belongs to the cytokine receptor superfamily. The multisubunit IL-6 receptor complex consists of the IL-6Rα subunit which binds to IL-6 and the membrane associated glycoprotein gp130 which is a signal transducer. Unlike most other cytokine receptors, the IL-6Rα subunit can be activated by ligand binding in both its membrane bound and its soluble form. IL-6 induces heterodimerization between IL-6Rα and gp130, which in turn leads to homodimerization of gp130 to a second gp130 molecule (see e.g. Hirano, T. (1998), Interleukin 6 and its receptor: ten years later. Int. Rev. Immunol. 16, 249-284). Actually, IL-6/IL-6Rα complexes can be potent activators of gp130, including in cells that lack membrane bound IL-6Rα. Since gp130 can be activated by several other ligand-receptor complexes, these effects may not reflect the physiological role of IL-6 (see e.g. Schirmacher, P., et al. (1998), Hepatocellular hyperplasia, plasmacytoma formation, and extramedullary hematopoiesis in interleukin (IL)-6/soluble IL-6 receptor double-transgenic mice. Am. J. Pathol. 153, 639-648). On the other hand, the fact that several different types of cytokine receptors can activate gp130 opens the possibility that different cytokines may potentiate each others actions thereby exerting synergistic effects. One example of receptors belonging to the IL-6Rα family is

the leptin receptor (Tartaglia, L. A. et al., (1995), Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263-1271) but the leptin receptor is not acting via gp130 (see e.g. Baumann, H., (1996), The full-length leptin receptor has signaling capabilities of interleukin 6-type receptors. Proc Natl. Acad. Sci. USA 93, 8374-8378).

Most patents issued regarding IL-6 have described methods to get beneficial effects of suppression of IL-6 action. One exception is a recent patent claiming that IL-6 can suppress demyelination, e.g. during multiple sclerosis (see US Pat. No. 5,863,529) Methods have been developed for production of human IL-6 in large quantities (see e.g. US Pat. No. 5,641,868).

Interleukin-6 agonists

Several IL-6 have been described in previous patent applications. For instance, possible superagonists made from wild type human IL-6 with various amino acid substitutions have been described (see e.g. US Pat. No. 5,914,106, US Pat. No.5,506,107, and US No. 5,891,998).

Interleukin-6 and obesity

It has recently been discovered that knockout of the IL-6 gene in mice surprisingly induces "middle age onset" obesity (Wallenius V and Jansson JO, unpublished results). There is little data in the literature indicating that IL-6 has any effect on metabolic parameters in the absence of acute phase reaction and inflammation. However, there are recent reports indicating that IL-6 is released from normal adipose tissue in humans. In addition, the IL-6 levels in blood are proportional to body fat mass (Mohamed-Ali V *et al.*, (1997), Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab* 82, 4196-200). If Il-6 prevents obesity, this finding suggest that obese individual could be IL-6 resistant,

and therefore benefit from treatment with a factor that enhances the effect of IL-6 in addition to IL-6 itself. In addition, it is well known that IL-6 is released from immune cells including macrophages, as well as endothelial cells and various other cell types (Hirano T, 1998). Moreover, both IL-6 and IL-6 receptors have been found in hypothalamic nuclei known to be important in the regulation of food intake and body weight (Schöbitz B *et al.*, 1993, see Fig. 1). These observations have drawn our attention to IL-6's potential role in the regulation of body weight.

IL-6 and acute phase reaction (APR)

IL-6 plays a role for different parts of the immune response (see e.g. Hirano, T. (1998), supra). It is well known that production of IL-6 as well as the circulating levels of this cytokine is enhanced during so-called acute phase reaction (APR). Moreover, IL-6 is considered a key mediator of APR, especially after infection with gram positive bacteria (see e.g. Kopf, M., et al. (1994), Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 368, 339-342). The APR is characterized by changes in the composition of the proteins released into plasma from the liver. APR is seen in pathological conditions with an inflammatory component such as trauma, infections, autoimmune disease, and tumors. These conditions are also associated with catabolism, i.e. decreased growth and increased degradation of tissues belonging to the fat free mass in the body.

IL-6 and ageing

Aging is associated with several somatic changes including increased body fat mass in general and visceral fat mass in particular (see e.g. Rudman, D., et al., (1990), Effects of human growth hormone in men over 60 years old. N. Engl. J. Med. 323, 1-6; Flier, J. S. and Foster D. W. (1998) supra). The proportion of the popula-

tion that have disturbances of blood fats such as pathologically elevated serum triglycerides also increase with age and is higher in middle aged than in young adult persons (Brown, M. S., and Goldstein, J. L. (1983) Disorders of lipid metabolism, Harrison's principle of internal medicine, 10th Ed, 547-559. It has been suggested that several age-associated diseases are caused by enhanced IL-6 (see e.g. Ershler, W. B., et al., (1994), The role of interleukin-6 in certain age-related diseases. Drugs Aging 5, 358-365). In humans there is an epidemiological connection between high IL-6 levels in peripheral blood mononuclear cells (PBMC) (see e.g. O'Mahony, L., et al., (1998), Quantitative intracellular cytokine measurement: age-related changes in proinflammatory cytokine production. Clin. Exp. Immunol. 113, 213-219) as well as in serum (see e.g. Mysliwska, J., et al., (1998), Increase of interleukin 6 and decrease of interleukin 2 production during the aging process are influenced by the health status. Mech. Aging Dev. 100, 313-328).

Effects of low, normal levels of IL-6 in mice of different age

There is much information about the effects of high levels of IL-6, e.g. in connection with inflammation (see e.g. Kopf, M., et al, supra). However, little is known about the importance of the low, basal levels of IL-6 in animals and humans without inflammation. One reason could be that it has been difficult to measure the low IL-6 levels in healthy mice with the assays available today. However, it can not be excluded that there still is a biologically significant effect of IL-6 in these animals. Moreover, IL-6 that is produced locally in tissues may exert autocrine or paracrine effects on cells in the same tissue, without being transported to other organs via blood circulation.

There have been few reports of differences between mice with complete IL-6 deficiency due to targeted dis-

ruption of the IL-6 gene, and normal wild type mice in the absence of provocations (see e.g. Hirano, T. (1998), supra). It is known that these mice develop normally to adulthood and they are fertile (see e.g. Kopf, M., et al, supra, and Poli, V., et al., (1994). Interleukin-6 deficient mice have been reported to be protected from bone loss caused by estrogen depletion. EMBO J. 13, 1189-1196). It has also been reported that IL-6 mice might have a defective fever response (see e.g. Hirano, T. (1998), supra). However, very little has been published about the effects of IL-6 deficiency in mice that are older than a couple of months. This could be due to the fact that it is expensive and laborious to keep mice for longer time. Since the normal life span of a mouse is about two years, there are few publications about a large part of the adult life of mice.

Regulation of IL-6 production and release

As mentioned above, IL-6 is released during acute phase reaction. Therefore, it is not surprising that IL-6 production is enhanced by gram-positive as well as by gram-negative bacteria. The latter seem to release IL-6 via production of an antigen called lipopolysaccharide (LPS) (see e.g. Kopf, M., et al. (1994), supra). The production of IL-6 is enhanced by tumor necrosis factor-α, TNF-α, a cytokine that is thought to play a role for the induction of type 2 diabetes, an illness associated with visceral obesity and cardiovascular disease. TNF-α production is enhanced from adipocytes that have accumulated fat (see e.g. Hotamisligil G. S. and Spiegelman B. M., (1994), Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes 43, 1271-1278; Flier, J. S. and Foster D. W. (1998), supra).

Several other hormones have also been shown to enhance IL-6 production. These include parathyroid hormone (PTH), 1,25-dihydroxyvitamin D3, thyroid hormone, platelet-derived growth factor, insulin-like growth factor I,

and IL-1 (see e.g. Swolin, D., et al., (1996), Growth hormone increases interleukin-6 produced by human osteoblast-like cells. J. Clin. Endocrinol. Metab. 81, 4329-4333, and references therein). In addition, it has been shown that nicotine, a well known suppresser of obesity, can enhance IL-6 production and plasma IL-6 levels (see e.g. Song, D-K.,et al., (1999), Central injection of nicotine increases hepatic and splenic interelukin-6 (IL-6) mRNA expression in mice: involvement of the peripheral sympathetic nervous system. FASEB J13:1259-1267). It has also been reported that corticosteroids, which are well known inducers of visceral obesity, can suppress IL-6 expression (see e.g. Swolin-Eide, D., et al., (1998), Effects of cortisol on the expression of interleukin-6 and interleukin-1 beta in human osteoblast-like cells. J. Endocrinol. 156, 107-114).

IL-6 and body fat during APR

IL-6 is a major mediator of APR, a condition associated with wasting and decreased appetite. However, it is still by no means certain that IL-6 also causes these anorectic and wasting effects. In fact, there are data indicating that this is not the case, although lipopolysaccharides (LPS) were reported to induce weight loss in mice and that this effect can be significantly prevented by treatment with anti-IL-6 monoclonal antibodies. However, in the same study the anti-IL-6 antibodies did not prevent the hypertriglyceridemia induced by LPS, possibly suggesting that IL-6 is less important for changes in fat metabolism during APR (Strassman, G. et al. (1993), The role of interleukin-6 in lipopolysaccharide-induced weight loss, hyperglycemia and fibrinogenproduction. Cytokine 5, 285-290).

It has been reported that IL-6 treatment can decrease lipoprotein lipase (LPL) activity in adipose tissue of mice and in murine adipocyte cell lines in vitro. This effect has been seen as an indication of a lipolytic

effect of IL-6 during cancer cachexia, a condition associated with APR (see Greenberg, A. S., (1992), Interleukin-6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes: a possible role for interleukin-6 in cancer cachexia, Cancer Res. 52, 4113-4116). On the other hand, there are indications e.g. from studies of gene knockout mice that LPL activity does not affect fat accumulation (Zechner, R (1997), The tissue specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism, Curr. Oppin. Lipidol. 877-88).

IL-6 and body fat during normal conditions

It has been speculated that that IL-6, like leptin, could have an adipostatic activity also in patients without APR. However, this assumption was based only on the finding that subcutaneous fat releases IL-6 in patients without acute phase reaction. Not surprisingly, there was also a correlation between high BMI, presumably reflecting fat mass, and levels of circulating IL-6 (Mohamed-Ali, V., et al. (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, in vivo, J. Clin. Endocrinol. Metab. 82,4196-4200). However, the finding that IL-6 is released by adipose tissue, does in no way prove that this factor would regulate fat tissue mass. As noted above, it is by no means clear that IL-6 is of importance for lipolysis even during APR. In the absence of APR, the available data has suggested that long term treatment with IL-6 in low, physiological doses is not lipolytic by itself. Although a single injection of IL-6 in a dose of 50 µg/kg body weight has been shown to enhance release of free fatty acids into blood circulation (Nonogagi K, et al. (1995), Interelukin-6 stimulates hepatic triglyceride secretion in rats, Endocrinology 136, 2143-2149), there is no obvious loss of fat mass in transgenic mice with very high levels of circulating IL-6 (see e.g. Peters, M. (1997), Extramedullary expan-

sion of hematopoietic progenitor cells in interleukin (IL)-6-sIL-6R double transgenic mice. J. Exp. Med. 185,755-766), although such mice display growth impairment (De Benedetti, F. et al. (1997), Interleukin-6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-1. J. Clin. Invest. 99, 643-650) as well as muscle atrophy (Tsujinak, T et al. (1996), Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice. J. Clin. Invest. 97, 244-249). Moreover, there have been few indications in the literature that long term absence of the low physiological amounts of endogenous IL-6 that are produced in an animal or human without APR, would have consequences for fat metabolism, especially fat mass and blood fat levels. The best way to investigate the consequences of long term absence is probably the study of mice with IL-6 gene knock out. In 1998 one of the worlds leading experts on IL-6 concluded in a review that the results of IL-6 knock out in mice had shown "that IL-6 is critical in only a limited range of biological reactions such as APR, the mucosal IgA response, the fever response, and estrogen deficiency-induced bone loss." (see e.g. Hirano, T. (1998), supra, p 252). No effects of fat mass in IL-6 knock-out mice have been reported. As noted above, IL-6 can suppress LPL (see Greenberg, A. S., (1992), supra), and it has also been suggested that LPL can increase predisposition for obesity and fat accumulation. On the other hand, this theory is challenged by the fact that fat specific deletion of LPL activity does not affect fat mass (Zechner, R (1997), The tissue specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism, Curr. Oppin. Lipidol. 8,77-88). The general opinion by well renowned researchers today is that IL-6 does not affect fat mass essentially, especially not during normal life without APR.

IL-6 and ethanol

Under certain circumstances, alcohol can suppress the concentration of circulating IL-6 (see e.g. Akerman, P. A., et al. (1993), Long-term ethanol consumption alters the hepatic response to the regenerative effects of tumor necrosis factor-alpha. Hepatology 17, 1066-1073). It is also well known that ethanol can cause visceral obesity as well as deranged blood fats including enhanced serum triglyceride levels (Brown, M. S., and Goldstein, J. L. (1983), supra).

TNF-α and regulation of body fat

As mentioned above, TNF-α is a stimulator of IL-6 production. This effect of TNF-α is exerted via the type 1 (p55) receptor, since it has been shown that IL-6 levels are decreased in mice with TNF receptor 1, but not TNF receptor 2, gene knock out (Yamada, Y., et al. (1998), Analysis of liver regeneration in mice lacking type 1 or type 2 tumour necrosis factor receptor: requirement for type 1 but not type 2 receptor. Hepatology 28,959-970). The role of TNF-α for development of obesity is not clear. Mice lacking the TNF-α ligand have not been reported to be obese (Uysal, K. T., et al (1997), Protection from obesity induced insulin resistance in mice lacking TNF-α, Nature 389,610-614), and there was no obesity in mice deficient in the both of the two receptors, type 1 (p55) and type 2 (p75), that are thought to mediate the biological effects of TNF-α. Actually, mice deficient in the type 2 (p75) receptor gain less weight when given high fat diet, suggesting that TNF-α might even stimulate obesity via this receptor type (Schreyer, S. A. (1998), Obesity and diabetes in TNF-α receptor deficient mice. J. Clin. Invest. 102,402-411). Furthermore, no increase in body weight was found in mice with TNF receptor 1 gene knock out even when they were fed high fat diet (Schreyer, S. A. (1998), supra). Obesity in *db/db (diabetes/diabetes)* mice with a defective leptin recep-

tor, was not affected by lack of the TNF receptor 1 (Schreyer, S. A. et al (1998), supra) or by lack of the ligand TNF-α which activates both receptor 1 and receptor 2 (Uysal, K. T., et al., (1997), supra). Another finding that argues against beneficial effects of TNF-α in obesity is that TNF-α often enhances insulin resistance, a symptom often associated with obesity (see Flier, J. S. and Foster D. W. (1998), supra).

Cytokines and atherosclerosis

Although the interest in the possible associations between cytokines and atherosclerosis has increased during recent years, it has mostly concerned the possible deleterious effects of cytokines and inflammation in development of atherosclerosis The cytokines have been assumed to stimulate the development of the atherosclerotic plaques by local effects (see e.g. Rus, H. G., et al., (1996) Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. Atherosclerosis 127,263-271). In addition, as mentioned above, IL-6 has been reported to increase circulating triglycerides by release of triglycerides from the liver (Nonogagi K, et al. (1995), Interleukin-6 stimulates hepatic triglyceride secretion in rats, Endocrinology 136, 2143-2149).

Summary of the invention

The object of the present invention is to provide new medicinal products and methods for treatment of obesity and/or obesity associated disorders.

The invention relates to the use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist for the production of a medicinal product for the treatment of obesity and/or obesity associated disorders.

Furthermore, the invention relates to a method for treatment of obesity and/or obesity associated disorders

wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist is administered to said patient.

The characterizing features of the invention will be evident from the following description and the appended claims.

Detailed description of the invention

In the research work leading to the present invention it was found that endogenous IL-6 can inhibit development of "middle-aged"-onset obesity as well as obesity associated disorders, e g the metabolic syndrome. The metabolic syndrome (also called syndrome X) comprises obesity (in particular abdominal obesity), disturbances of blood fats (e g triglycerides), and diabetes type II.

The invention thus relates to medicinal products comprising a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist. Said substance may be an IL-6 receptor agonist. A preferred example of such an agonist is IL-6. It is possible to use a naturally occurring agonist, such as IL-6, as well as a synthetically produced agonist, such as an IL-6 mimetic. Examples of synthetically produced IL-6 receptor agonists are given in US 550 61 07 (Cunningham et al), US 589 19 98 (Rocco et al), and US 591 41 06 (Gennaro et al). Said substance may also be a substance that upon administration will lead to the release of an endogenous occurring IL-6 receptor agonist, preferably IL-6, from different cells, such as endothelial cells, or organs, such as the liver.

The expression "IL-6 receptor agonist" used herein relates to all substances that bind to and activate the same receptor proteins as IL-6.

The term "patient" used herein relates to any human or non-human mammal in need of treatment with the medicinal product or method according to the invention.

The term "treatment" used herein relates to both treatment in order to cure or alleviate a disease or a condition, and to treatment in order to prevent the development of a disease or a condition. The treatment may either be performed in an acute or in a chronic way.

As mentioned above, the invention is suitable for treatment of high levels of triglycerides. The expressions "high levels of triglycerides" relates to amounts of this compound that are higher than for a normal, healthy person.

The medicinal product and the method according to the invention are suitable for treatment of different pathological disturbances of regulation of body fat tissues, leading to obesity and/or obesity associated disorders. One example is visceral or general obesity that is due to genetic predisposition, a condition sometimes described as the thrifty genotype. Another example is diet-induced obesity, a condition that often is resistant to leptin treatment.

The medicinal product and the method according to the invention are e.g. suitable for treatment of cardiovascular disease, since obesity and obesity associated disorders are associated with an increased risk of cardiovascular disease.

The medicinal product and the method according to the invention are also suitable for treatment of persons that have been exposed to high doses of glucocorticoid hormone, e.g. due to tumours producing such hormones, due to treatment with glucocorticoids against certain diseases, or due to abuse of glucocorticoids. It is known that high levels of glucocorticoids cause visceral obesity and disturbed blood fats. It has been shown that glucocorticoids under certain circumstances can decrease IL-6 production.

Other patients which may be treated with the medicinal product or the method according to the invention are persons with obesity, obesity associated disorders,

and/or low endogenous production of IL-6 during normal state, i.e., in the absence of APR. Also persons with obesity and/or obesity associated disorders in combination with insensitivity to IL-6 may be treated with the medicinal product and the method according to the invention. The IL-6 insensitivity could e.g. be caused by low levels of the receptor protein IL-6Rα on the cell surface or low levels of the glycoprotein gp130 which normally mediates the effects of IL-6. In these persons, the IL-6 produced by the patients themselves may not be sufficient to inhibit development of obesity and/or obesity associated disorders.

Another example of a group of patient which may be treated according to the invention are patients suffering from normal aging. In some cases, the production of IL-6 in important tissues could be insufficient although the circulating levels often are increased. A possible IL-6 insufficiency in aging may also be due in part to insensitivity to IL-6.

It is also possible to treat patients with obesity and/or obesity associated disorders in combination with low concentrations of growth hormone (GH) receptors or defective GH receptors. It is known that GH has lipolytic effects.

It is also possible to treat obese patients with low concentrations of leptin or leptin receptors, or patients with defective leptin receptors. More often, it would be beneficial to treat patients with obesity and/or obesity associated disorders in combination with leptin resistance due to unknown reasons.

Also patients abusing alcohol may suffer from conditions treatable according to the present invention. It has been shown that alcohol may decrease IL-6 levels (Akerman, P. A., et al. (1993), supra) and that patients abusing alcohol often display increase visceral obesity and enhanced serum triglyceride levels in man (Flier, J. S. and Foster D. W. (1998), supra).

It may be advantageous to combine the substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist used according to the invention with a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist, and the medicinal product according to the invention may thus also comprise such a factor. An example of such a factor is a soluble IL-6 binding protein. However, a problem may be that IL-6 in combination with soluble IL-6Rα may exert unspecific effects, including even on cells that do not have membrane bound IL-6Rα (see e.g. Peters, M. (1997), supra).

The medicinal product according to the invention may also comprise other substances, such as an inert vehicle, or pharmaceutical acceptable adjuvants, carriers, preservatives etc., which are well known to persons skilled in the art.

The medicinal product according to the invention may be formulated for enteral (e.g. oral or per oral) or parenteral administration.

The invention also relates to use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist for a medicinal product for treatment of the above specified conditions.

Furthermore, the invention relates to a method for treatment of pathological disturbances of fat metabolism wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist is administered to said patient. Preferably, said substance is administered together with a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist.

Since these effects of IL-6 on fat metabolism were first seen in the work leading to the present invention after removal of endogenous IL-6, it seems appropriate to

use IL-6 according to the invention in doses that previously have been used to substitute for IL-6 deficiency. Such a dose would be about 1 mg/kg body weight given as a subcutaneous injection to mice (se e.g. e.g. Cressman, D. E., et al., (1996), Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 274, 1379-1383). However, the dose of IL-6 in humans could be quite different. The dose may be higher in older individuals, since it has been shown that IL-6 levels increase with age. The dose may be lower than those doses that would result in IL-6 levels found during APR, to avoid side effects similar to the symptoms of APR.

The invention will now be further explained in the following examples. These examples are only intended to illustrate the invention and should in no way be considered to limit the scope of the invention.

Brief description of the drawings

In the examples below reference is made to the accompanying drawing on which:

Fig 1 A shows the effect of interleukin-6 gene knock out in male mice on mean body weight at different ages. Fig 1 B shows the physical appearance of IL-6 knock out male mice at 9-10 months of age. The photo shows representative body shapes of IL-6 -/- and IL-6 +/+ male mice. The computerized tomography (CT) shows transverse sections of the abdomen of representative IL-6 -/- and IL-6 +/+ male mice (C).

Fig 2 A, B and C illustrates the effects of interleukin-6 gene knock out on mean body weight at different ages in female mice (Fig 2 A) and the effect of interleukin-6 gene knock out on mean body mass index (Fig 2 B) (BMI, body weight/(crown-rump length)^2) and mean visceral transversal width (mm) (Fig 2 C) were also investigated in 9 month-old female mice.

Fig 3 Shows the measured daily food intake during three consecutive days in 11 month-old female IL-6 +/+ and IL-6 -/- mice.

Fig 4 A and B illustrates the effects of interleukin-6 gene knock out in female mice on serum triglyceride levels (Fig 4 A) and serum leptin levels (Fig 4 B).

Fig 5 shows the possible sources of IL-6 that could be of importance for body composition and leptin sensitivity.

Fig 6 shows the effect of vehicle and leptin administration on food intake in 15 month-old wild-type and IL-6 knockout (IL-$6^{-/-}$) male mice. 8 A shows vehicle treated mice, wild-type n = 5, IL-$6^{-/-}$ n = 4. 8 B shows leptin at 120 µg/day, n = 5 per genotype. 8 C shows leptin at 240 µg/day, wild-type n = 5, IL-$6^{-/-}$ n = 3. Thick black bars represent leptin treatment period. Vehicle or leptin was injected intraperitoneally twice daily. Values are indicated as mean ± SEM. # P<0.05, ## P<0.01, ### P<0.001 vs. study day 0, paired t test with the Bonferroni correction. * P<0.05, ** P<0.01 vs. wild-type, independent t test.

Fig 7 shows the effect of vehicle and leptin administration on body weight in 15 month-old wild-type and IL-6 knockout (IL-$6^{-/-}$) male mice. 9 A shows vehicle treated mice, wild-type n = 5, IL-$6^{-/-}$ n = 4. 9 B shows leptin at 120 µg/day, n = 5 per genotype. 9 C shows leptin at 240 µg/day, wild-type n = 5, IL-$6^{-/-}$ n = 3. Thick black bars represent leptin treatment period. Vehicle or leptin was injected intraperitoneally twice daily. Values are indicated as mean ± SEM. # P<0.05, ## P<0.01, ### P<0.001 vs. study day 0, paired t test with the Bonferroni correction. * P<0.05, ** P<0.01, *** P<0.001 vs. wild-type, independent t test.

Fig 8 shows relative weights of different fat depots (% fat weight/body weight) in IL-$6^{+/+}$ and IL-$6^{-/-}$ mice. Three intra-abdominal fat pads (gonadal, retroperitoneal and mesenteric) and the femoral fat pad

(a subcutaneous fad pad on the outer thigh) were dissected and weighed in 18-month-old male (A) and female (B) IL-6+/+ and IL-6-/- mice. There were 4-10 mice in each group. * P < 0.05, ** P < 0.01 and *** P<0.001, vs. corresponding IL-6+/+ mice.

Fig 9 shows comparison of the effect of IL-6 treatment in IL-$6^{+/+}$ and IL-$6^{-/-}$ mice. The mice were treated with gradually increasing doses of IL-6 (40 ng/day, days 0-4; 80 ng/day, days 5-12; 160 ng/day, days 13-18). Changes in body weight (g) during the IL-6 treatment period compared to before start of treatment (A). Figures 11 B and C compare values at day 0 before initiation of IL-6 treatment with day 18 after IL-6 treatment in IL-$6^{+/+}$ and IL-$6^{-/-}$ mice. The total abdominal area was calculated from the CT scans (B). The intraperitoneal area containing fat was measured separately by calculating the darker areas with attenuation similar to subcutaneous fat (C). Both the total intraperitoneal and intraperitoneal fat areas were calculated blindly by two different people, with no connection to the study. There were 5 mice in each group. All animals were 12-month-old at the start of the treatment. * *P* < 0.05, ** *P* < 0.01 and *** *P*<0.001, vs. corresponding control mice. # P <0.05, vs. the corresponding group before initiation of IL-6 treatment.

Examples

The IL-6 knock out mice (i.e. IL-6 -/- mice) and the corresponding controls used in these examples were kindly provided by Dr. Manfred Kopf at Basel Institute of Immunology, Basle, Switzerland (see e.g. Kopf, M. (1994), supra). The IL-6 -/- mice were back crossed 7-8 times with c57Bl/6 mice to gain a strain of mice genetically consisting of more than 95 % c57Bl/6.

As controls to the IL-6 -/- mice, wild type c57Bl/6 mice (i.e. IL-6 +/+ mice) (Bomholtgård Breeding & Research Centre A/S) were used in examples 1-4. These mice were kept at standardized conditions with standard low

fat chow and water freely available. Food intake was measured keeping two female mice per cage. The amount of chow was recorded once per day. In example 5 age-matched normal C57BL/6 male mice from B&K Universal AB (Sollentuna, Sweden) were used as wild-type controls. All male mice were housed separately (due to aggressiveness) in standard cages under standardised environmental conditions, *i.e.* 24-26°C, 50-60% relative humidity, artificial lightning at 05:00-19:00 hours, with water and pelleted food (Beekay Feeds, Rat and mouse standard diet, B&K Universal AB, Sollentuna, Sweden) *ad libitum*.

In examples 6 and 7, mice with IL-6 gene knock-out (IL-$6^{-/-}$ mice) were generated as described by Kopf *et al* (12). To reduce genetic heterogeneity, the IL-$6^{-/-}$ genotype was moved onto C57BL/6 background by eight successive back crosses. The resulting strain of mice consists genetically of more than 99.5% C57BL/6. Normal C57BL/6 mice from B&K Universal (Sollentuna, Sweden) were used as wild-type controls for the IL-$6^{-/-}$ mice. The animals were maintained under standardized environmental conditions, *i.e.* 24-26°C, 50-60% relative humidity, artificial lighting at 05.00-19.00 h, with water and pelleted food *ad libitum*. All procedures regarding the mice were conducted in accordance with protocols approved by the institutions (Göteborg and Lund) and the local ethical committees on animal care.

Measurements of body weight and food intake

In examples 1-4 the body weight of the IL-6 -/- mice and wild type control female mice were recorded regularly. The crown-rump length and the transversal abdominal diameter were measured in anesthetized animals by dual x-ray absorptiometry (DEXA) using the Norland pDEXA Sabre (Fort Atkinson, WI, USA). Body mass index was then calculated for each mouse as body weight/crown-rump length^2. Visceral and subcutaneous obesity was also evaluated by computerized tomography (CT) at a level 5 mm

cranially of the junction between the L6 and S1 vertebras.

In example 5 body weight was measured using a weighing scale (A & D Instruments, EK-200G). Food consumption was measured daily by weighing the food left over 24 h after the previous fillup. Basal food intake was measured during pre-treatment with saline injections before onset of the leptin treatment. Body weight and food intake was measured for 3 days after the end of leptin treatment.

Leptin measurement

Plasma leptin was determined with a recently described radioimmunoassay (Ahrén, B. et al. (1997) Regulation of plasma leptin in mice: Influence of age, high-fat diet and fasting, Am. J. Physiol. 273, R113-R120; Linco Research, St Charles, Mo, USA). The method uses a polyclonal rabbit antibody raised against recombinant mouse leptin, ^{125}I-labeled tracer prepared with recombinant mouse leptin and mouse leptin as standard. Rabbit anti-rabbit IgG was used for separation of bound and free leptin.

In example 5 tail blood samples were collected from young (4 months) and old (12 months) wild-type and IL-6 $^{-/-}$ male mice.

Differences between IL-6 -/- and IL-6 +/+ control mice were determined by Student's t-test. When more than two groups were compared, statistics were calculated by one-way ANOVA followed by Student-Newman-Keuls multiple range test.

Example 1

IL-6 -/- knock-out male mice were not heavier than their wild type littermates at 2-5 months of age. However, the body weight of 9 months old IL-6 -/- male mice was higher than that of the corresponding wild type animals, as evident from Fig. 1 A. The physical appearance of male mice at 9-10 months of age clearly showed that

the IL-6 -/- mouse was considerably fatter than a wild type control of the same age, as shown in Fig. 1 B). Computerized tomography (CT) of the abdomen clearly indicated that both visceral (intraabdominal) and subcutaneous fat mass were markedly increased in the IL-6 -/- mice compared to the wild type control, as evident from Fig. 1 C.

Example 2

In this example the effects of IL-6 knock-out on body weight was studied at different ages in female mice. The body weight did not differ between wild type and knock-out female mice between two and five months of age, but between seven and nine months of age the body weight was significantly higher in IL-6 -/- than in wild type +/+ mice, as seen in Fig. 2 A. The body mass index of 9-10 months old IL-6 knock-out female mice was higher than that of the corresponding wild type females, which is illustrated in Fig. 2B. The transversal abdominal diameter, as measured by DEXA, was also larger in IL-6 knock-out female mice than in wild type controls at 9-10 months of age (Fig. 2C).

Example 3

Thereafter the daily food intake for three consecutive days was studied for 11 months old IL-6 -/- female mice compared to in wild type IL-6 +/+ controls. From the results shown in Fig. 3 it is clearly evident that the food intake was increased in the L-6 -/- mice compared to the controls.

Example 4

Serum triglyceride and cholesterol levels of 11 months old female IL-6 -/- mice were compared to wild type IL-6 +/+ controls. As can be seen in Fig. 4 A the serum triglyceride was considerably higher in the IL-6 -/- mice. Also the circulating levels of leptin were mark-

edly higher, i.e., about three times, compared to those of wild type mice, as seen in Fig. 4 B.

Example 5

15-month-old IL-6 $^{-/-}$ and wild-type males received intraperitoneal (ip) injections of leptin at doses of 120 µg/day or 240 µg/day or vehicle twice daily (at 08:30 and 17:00) for 3 consecutive days. Human leptin was obtained from PeproTech (Rocky Hill, NJ, USA) and dissolved in sterile PBS, 0.1% BSA. In order to get the animals used to injections, mice were given saline injections twice daily before the start of the leptin treatment.

The descriptive statistical results are presented as means ± SEM. Independent *t* test was used to test between-group differences. Within-group differences were analysed using paired *t* test followed by the Bonferroni correction. $P < 0.05$ was considered significant.

Effects of leptin treatment on food intake

Vehicle treatment (PBS, 0.1% BSA) showed no effect on food intake compared to baseline levels in wild-type and IL-6 $^{-/-}$ mice (Fig. 6 A).

In contrast, treatment with leptin at a dose of 120 µg/day to wild-type male mice led to a 40% decrease in food intake during the first two treatment days compared to baseline levels (baseline level: 4.91 ± 0.08 g). Food intake was not significantly decreased in IL-6 $^{-/-}$ mice during treatment with leptin in this dose (Fig 6 B). The decrease in food intake was significantly larger in wild-type mice than in IL-6 $^{-/-}$ mice on day 1-3 of leptin treatment (Fig 6 B). At the end of the leptin treatment, food intake was normalised within 2 days in wild-type mice.

Leptin treatment at a larger dose (240 µg/day) led to a reduction of food intake in wild-type males with the largest decrease (50%) from baseline level during the third treatment day (baseline level: 4.46 ± 0.30 g, Fig

8 C). There was no decrease in food intake in the IL-6 $^{-/-}$ mice (Fig 6 C). Three days after the end of the leptin treatment, food intake increased significantly to above baseline levels in wild-type mice and there was a similar tendency in IL-6 $^{-/-}$ mice (Fig 6 C).

Effects of leptin treatment on body weight

Vehicle treatment (PBS, 0.1% BSA) showed no effect on body weight compared to baseline levels in wild-type and IL-6 $^{-/-}$ mice (Fig 7 A).

However, body weights were markedly reduced during and after leptin treatment (120 µg/day) in wild-type mice, while the effect was less pronounced in the IL-6 $^{-/-}$ mice (Fig 7 B). The reduction in body weight was significantly larger in wild-type mice than IL-6 $^{-/-}$ mice day 1-4 after initiation of leptin treatment.

Body weights were significantly reduced in wild-type mice both for three days during and for three days after a higher dose of leptin treatment (240 µg/day, Fig 9 C). There was a tendency towards decreased body weights in leptin treated IL-6 $^{-/-}$ mice, but this decrease was not significant tested with paired *t* test followed by the Bonferroni correction for five comparisons. On day 3 of leptin treatment, the decrease in body weight was significantly smaller in IL-6 $^{-/-}$ mice than in wild-type mice.

Discussion

In has thus been shown that IL-6$^{-/-}$ mice have decreased responsiveness to leptin treatment compared to wild type mice. These findings indicate that presence of endogenous IL-6 is of importance for normal leptin responsiveness. Leptin treatment induced a significant reduction in food intake in the wild-type mice, but not in the IL-6 $^{-/-}$ mice. In addition, the suppressive effect of leptin on body weight was less pronounced in IL-6 $^{-/-}$ mice than in wild-type mice. These effects of IL-6 may be re-

lated to the IL-6 receptor structure, since it has been shown that IL-6 type cytokine receptors share functional specificity with the long form of the leptin receptors (Ob-Rb, Baumann H *et al.*, 1996). The receptor subunits for ciliary neurotrophic factor (CNTF) have been shown to share sequence similarities with Ob-Rb, Gloaguen I *et al.*, 1997) and IL-6 receptors. When administered systemically, CNTF can reverse obesity in various animal models, including *db* mice lacking leptin receptors (Gloaguen I *et al.*, 1997). All three of these systems, leptin, IL-6 and CNTF, signals through the JAK-STAT pathway to regulate gene expression (Flier JS, 1997; Hirano T, 1998; Gloaguen I *et al.*, 1997). Cross-reactivity between the three systems at the receptor or post-receptor level may serve as an explanation for the link between regulation of body weight by leptin and IL-6 (as well as CNTF).

It has also been shown that the body weights of the IL-6 $^{-/-}$ mice in this study were significantly higher compared with the body weights of wild-type mice. This result is supported by the recent finding that IL-6 $^{-/-}$ mice develop "middle age onset" obesity (Wallenius V and Jansson JO, unpublished results). There may be several possible reasons why the obese phenotype of these mice has not been noticed previously. IL-6 $^{-/-}$ mice are commonly used to investigate the role of IL-6 in various infectious and inflammatory models (Kopf *et al.* 1994), but the weight gain in the IL-6 $^{-/-}$ mice was not observed until they were "middle aged", that is about 4 months of age. Younger animals are preferred for studying infection and inflammation. Moreover, the IL-6 $^{-/-}$ mice in this study were back-crossed for 8 generations to a 99.5% pure C57BL/6 background, which may be of importance for the development of the obese phenotype. If so, this raises the question whether the obese phenotype is exclusive for IL-6 $^{-/-}$ mice with a C57BL/6 background or if it also would be seen in other mice strains deficient for IL-6.

The weight gain in the IL-6 $^{-/-}$ mice could be secondary to the development of leptin resistance indicated by this study. If this is the case, one could expect the IL-6 $^{-/-}$ mice to have a higher level of basal food intake compared to wild-type mice. So far, studies on basal food intake in IL-6 $^{-/-}$ mice have not shown such results. There are also indications in the literature, suggesting that IL-6 affects energy expenditure rather than feeding (Chrousos GP, 1995). If IL-6 acts mainly on the regulation of energy expenditure relative to the regulation of appetite/food intake, the finding in this study that endogenous IL-6 may potentiate the suppressive effect of leptin on food intake is a bit surprising (Friedman JM and Halaas JL, 1998). It is common knowledge that food intake and appetite is reduced during infectious diseases and inflammation, conditions which are associated with increased levels of circulating IL-6 (Hirano T, 1998). However, there have been few earlier indications that the low basal production of IL-6 in healthy animals would affect food intake or fat mass. So far, the reason for the weight gain in the IL-6 $^{-/-}$ mice is not clear and needs further investigation.

Measurement of plasma leptin levels in male IL-6 $^{-/-}$ mice and wild-type male mice showed no significant difference between old IL-6 $^{-/-}$ mice and old wild-type mice. This is surprising for two reasons. Firstly, the IL-6 $^{-/-}$ mice were heavier than the wild-type mice because of increased body fat mass (Wallenius V and Jansson JO, unpublished results). Since plasma leptin levels are highly correlated with adipose tissue mass (Friedman JM and Halaas JL, 1998), the plasma leptin levels of the IL-6 $^{-/-}$ mice were expected to be higher than in the wild-type mice. Secondly, leptin resistance in the IL-6 $^{-/-}$ mice, as indicated by this study, is associated with increased plasma leptin levels. For instance, elevation of plasma leptin is seen in most obese humans with leptin resistance (Flier JS and Foster DW, Williams textbook of endo-

crinology 9th edition). Other measurements of plasma leptin levels in female mice have shown increased levels in the IL-6 $^{-/-}$ mice compared to wild-type mice (Wallenius V and Jansson JO, unpublished results). It is known that the levels of circulating leptin are higher in females than in males (Flier JS and Foster DW, Williams textbook of endocrinology 9th edition), and there are several gender differences in the regulation of fat mass (Vettor R *et al.*, 1997). Therefore, the preliminary results of the measurements of plasma leptin levels in male IL-6 $^{-/-}$ mice need to be repeated and investigated further.

Example 6

In this example, the increase in body fat caused by IL-6 deficiency was confirmed by fat dissections in 18-month-old male (shown Fig. 8 A) and female (shown in Fig. 8 B) mice. Four different fat pads were dissected from these mice. The male and female IL-6$^{-/-}$ and IL-6$^{+/+}$ mice were first weighed and then three intra-abdominal fat pads (gonadal, retroperitoneal and mesenteric) and the femoral fat pad (subcutaneous pad in the groin of the thigh) were dissected and weighed. All investigated fat pads, except the male mesenteric fat pad (Fig. 8 A), were significantly larger in the IL-6$^{-/-}$ mice compared to IL-6$^{+/+}$ mice. In both males and females the total weight of all dissected fat pads was increased by 50-60 % in IL-6$^{-/-}$ compared to IL-6$^{+/+}$ mice (not shown).

Example 7

In this example female IL-6$^{-/-}$ and IL-6$^{+/+}$ mice were treated with IL-6 to see if it was possible to reverse some of the phenotypical changes observed in the IL-6$^{-/-}$ mice. Figure 9 A shows that 18 days of IL-6 treatment reduced body weight to a larger extent in IL-6$^{-/-}$ mice than in IL-6$^{+/+}$ mice. Quantification of several CT scans performed before the start of IL-6 treatment showed that the intraperitoneal area was significantly higher in the IL-

$6^{-/-}$ mice compared to the IL-$6^{+/+}$ (Fig. 9 B). After 18 days of IL-6 treatment the total abdominal area had decreased significantly in the IL-$6^{-/-}$ mice while there was no such effect in the IL-$6^{+/+}$ mice (Fig. 9 B). Intraperitoneal areas were also measured, and they had a similar attenuation on the CT scans as subcutaneous fat. This quantification, excluding non-fat tissues, indicated an even larger increase in the fat content in IL-$6^{-/-}$ mice compared to the IL-$6^{+/+}$ mice (Fig. 9 C). There was a significant decrease in the intraperitoneal areas with fat-like attenuation after IL-6 treatment to the IL-$6^{-/-}$ mice (Fig. 9 C). Before IL-6 treatment, leptin levels were almost three times higher in the IL-$6^{-/-}$ mice compared to the IL-$6^{+/+}$ mice. IL-6 replacement for 18 days to the IL-$6^{-/-}$ mice caused a significant decrease in leptin levels compared to before treatment.

The computerized tomographies (CTs) in this example were performed with the Stratec peripheral quantitative computerized tomography (pQCT) XCT Research M (software version 5.4B; Norland Medical Systems Inc., Fort Atkinson, WI) operating at a resolution of 70 μm. The section was made at the same point in all mice, *i.e.* 5 mm proximally of the *crista illiaca*.

CLAIMS

1. Use of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist for the production of a medicinal product for treatment of obesity and/or obesity associated disorders.

2. Use according to claim 1, wherein said substance is an IL-6 receptor agonist.

3. Use according to claim 2, wherein said substance is IL-6.

4. Use according to any one of the claims 1-3, wherein said obesity and/or obesity associated disorders is caused by a pathological disturbance of fat metabolism.

5. Use according to claim 4, wherein said obesity is mainly visceral or intraabdominal.

6. Use according to any one of the claims 1-5, wherein said obesity is observed despite high levels of circulating leptin.

7. Use according to any one of the claims 1-6, wherein said obesity is accompanied by leptin insensitivity.

8. Use according to any one of the claims 1-3, wherein said disorder is a pathological increase of serum triglycerides.

9. Use according to any one of the claims 1-8, wherein said medicinal product is suitable for treatment of a cardiovascular disease.

10. Use according to any one of the claims 1-8, wherein said medicinal product is suitable for treatment of the metabolic syndrome.

11. Use according to any one of the claims 1-8 or 10, wherein said medicinal product is suitable for treatment of diabetes type II.

12. Use according to any one of the claims 1-11, wherein said medicinal product is suitable for treatment of a condition due to ageing.

13. Use according to claim 12, intended for a human patient of the age 30 years or older.

14. Use according to any one of the claims 1-13, wherein said medicinal product further comprises a factor that will intensify the effect of said interleukin-6 (IL-6) receptor agonist.

15. Use according to claim 14, wherein said factor is a factor acting via gp130.

16. Use according to claim 14, wherein said factor is leptin.

17. A method for treatment of obesity and/or obesity associated disorders wherein a pharmaceutically effective amount of a substance that upon administration to a patient will lead to an increased level of an interleukin-6 (IL-6) receptor agonist is administered to said patient.

18. A method according to claim 17, wherein said substance is an IL-6 receptor agonist.

19. A method according to claim 18, wherein said substance is IL-6.

20. A method according to any one of the claims 17-19, wherein said obesity and/or obesity associated disorders is caused by a pathological disturbance of fat metabolism.

21. A method according to claim 20, wherein said obesity is mainly visceral or intraabdominal.

22. A method according to according to any one of the claims 17-21, wherein said obesity is observed despite high levels of circulating leptin.

23. A method according to according to any one of the claims 17-22, wherein said obesity is accompanied by leptin insensitivity.

24. A method according to any one of the claims 17-19, wherein said condition is a pathological increase of serum triglycerides.

25. A method according to any one of the claims 17-24, wherein said medicinal product is suitable for treatment of a cardiovascular disease.

26. A method according to any one of the claims 17-25, wherein said medicinal product is suitable for treatment of a condition due to ageing.

27. A method according to claim 26, wherein said patient is a human of the age 30 years or older.

28. A method according to any one of the claims 17-27, wherein said IL-6 receptor agonist is administered in combination with a factor that will intensify the effect of said IL-6 receptor agonist.

29. A method according to claim 28, wherein said factor is a factor acting via gp130.

30. A method according to claim 28, wherein said factor is leptin.

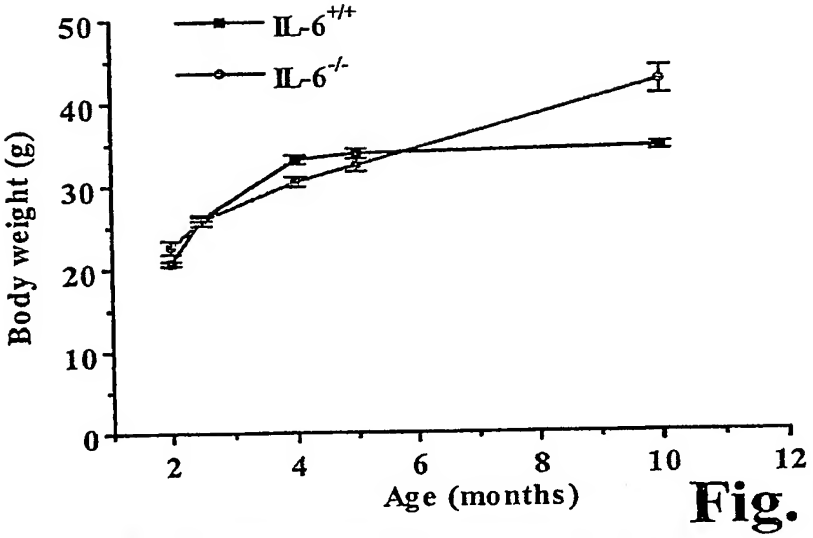

Fig. 1 A

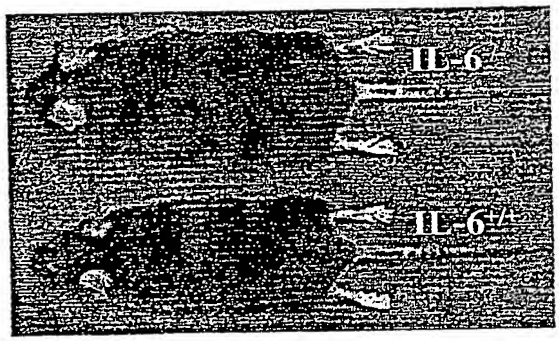

Fig. 1 B

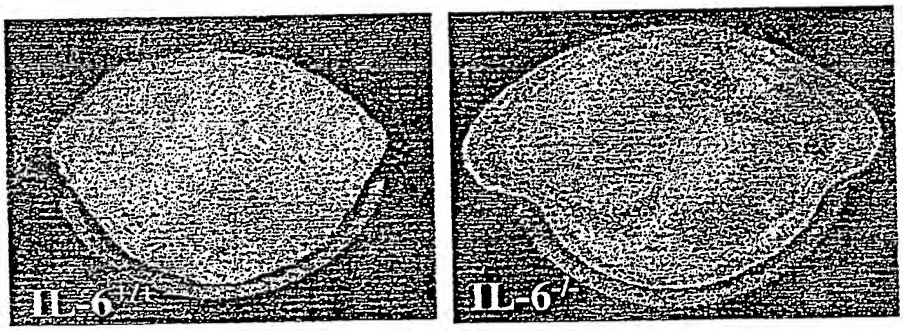

Fig. 1 C

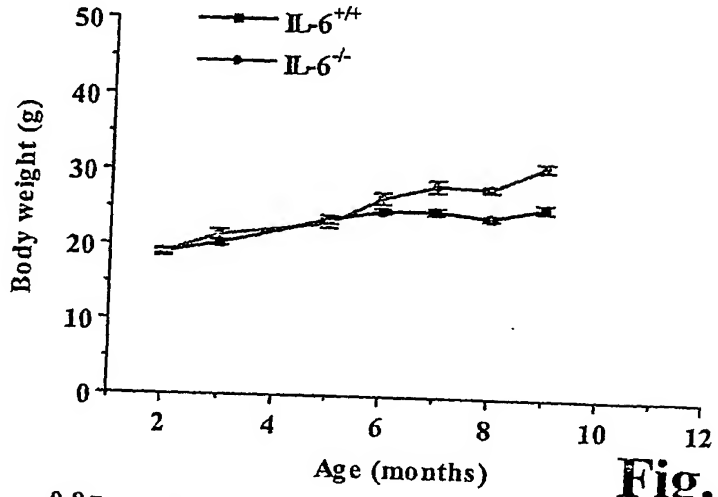

Fig. 2 A

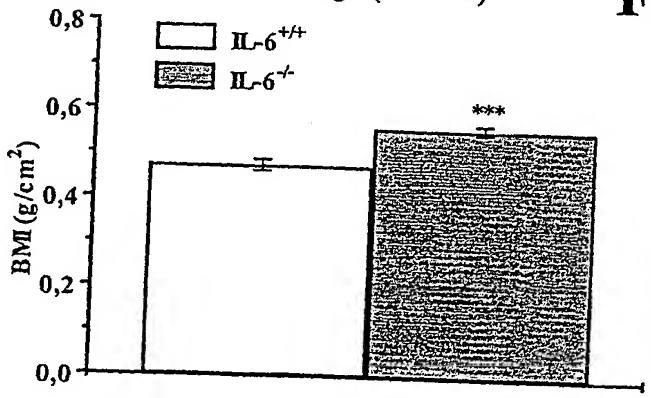

Fig. 2 B

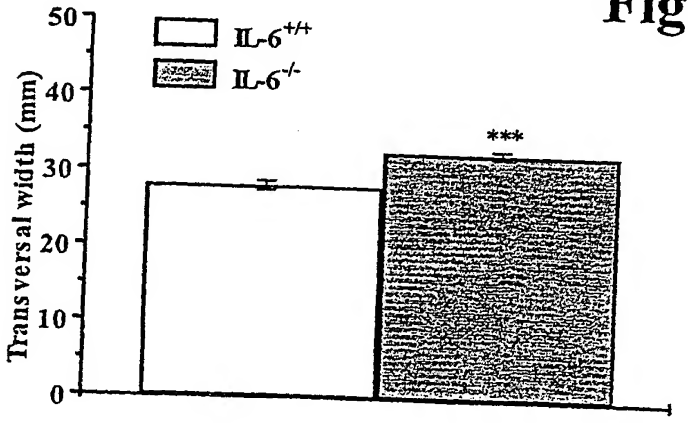

Fig. 2 C

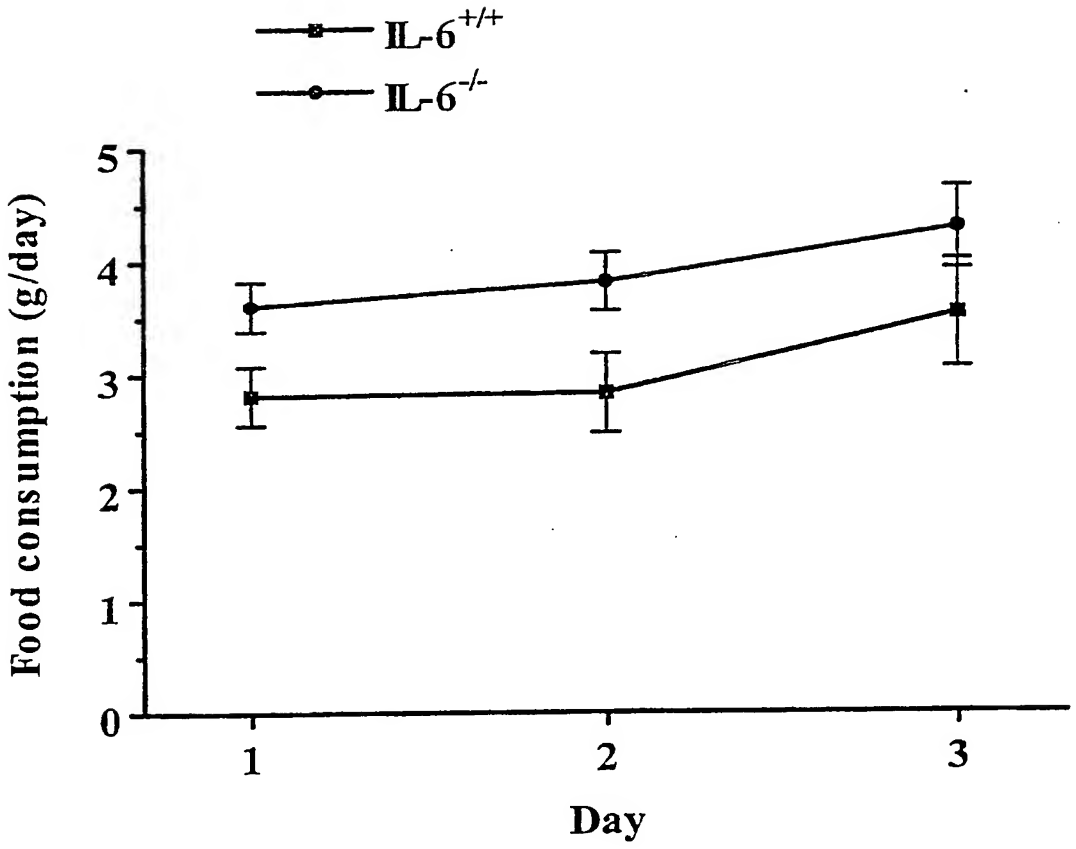

Fig. 3

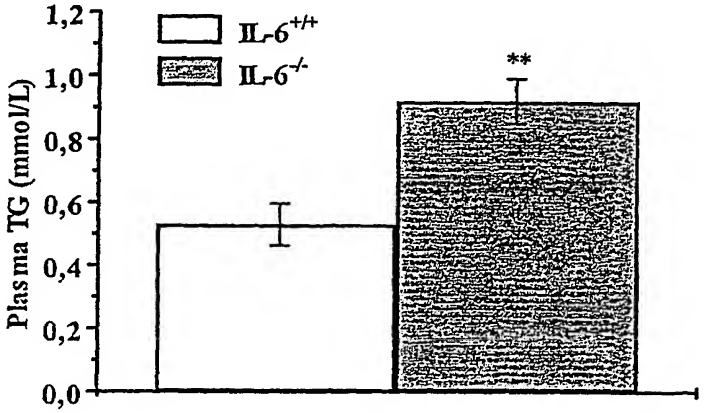
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Plasma TG (mmol/L)
IL-6+/+
IL-6-/-
**

Fig. 4 A

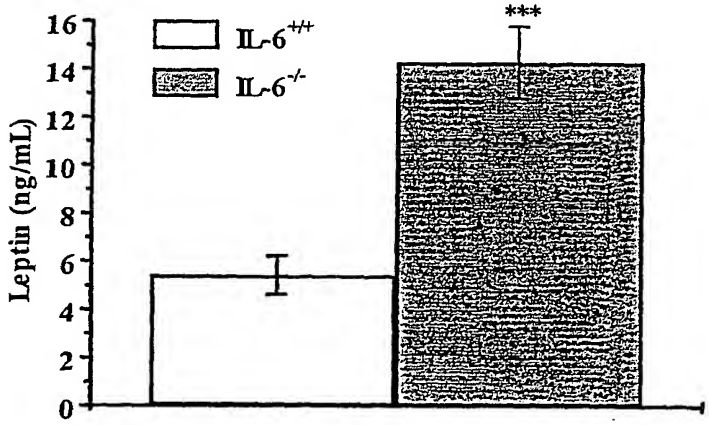
16
14
12
10
8
6
4
2
0
Leptin (ng/mL)
IL-6+/+
IL-6-/-
***

Fig. 4 B

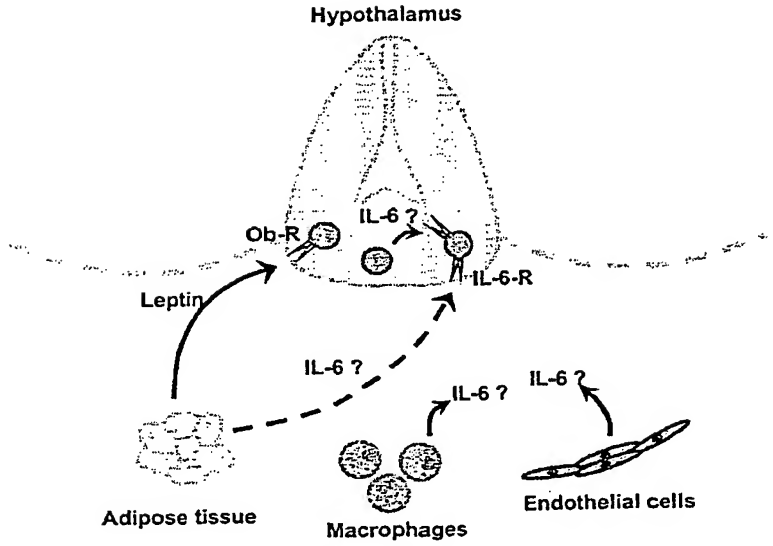
Hypothalamus
Ob-R
IL-6 ?
IL-6-R
Leptin
IL-6 ?
IL-6 ?
IL-6 ?
Adipose tissue
Macrophages
Endothelial cells

Fig. 5

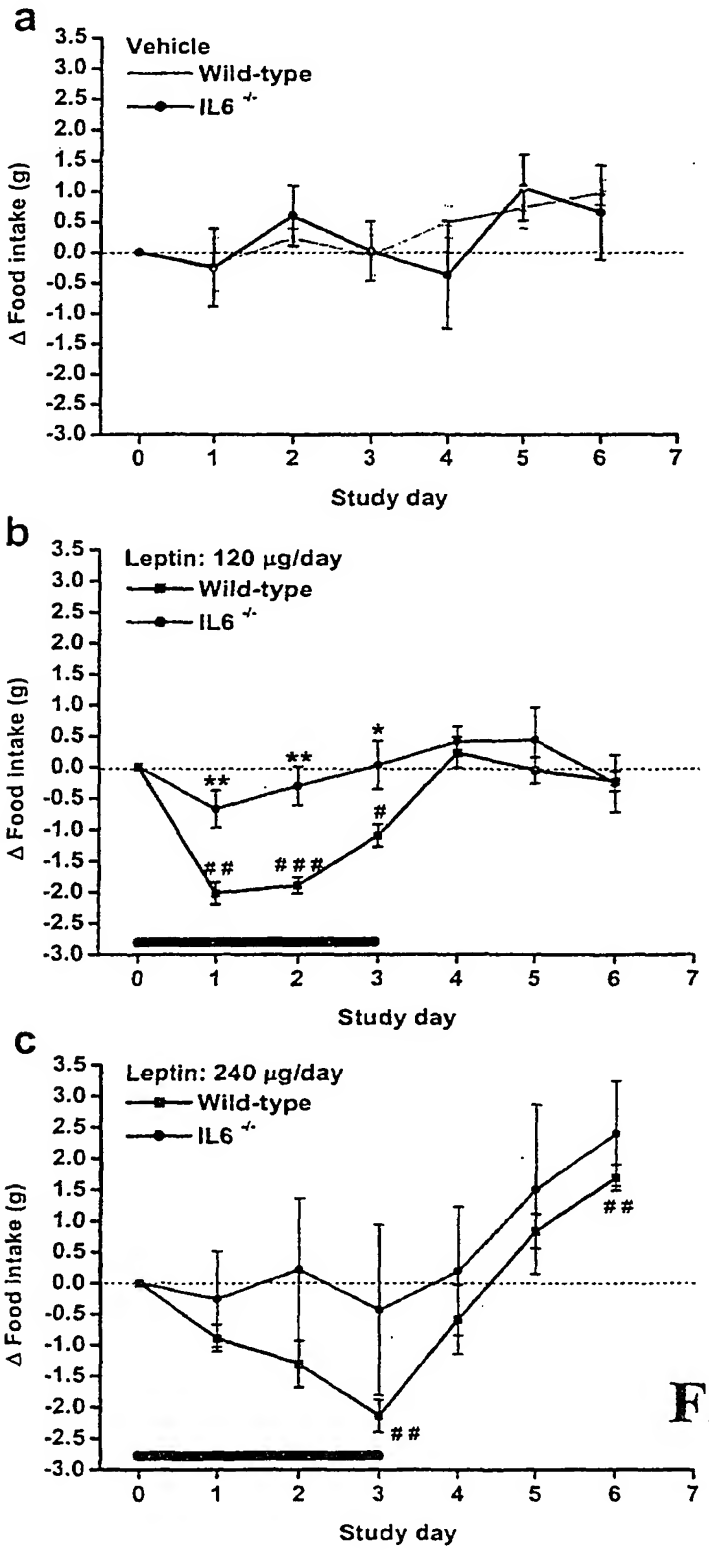
a
Vehicle
Wild-type
IL6 -/-
Δ Food intake (g)
Study day
b
Leptin: 120 µg/day
Wild-type
IL6 -/-
Δ Food intake (g)
Study day
c
Leptin: 240 µg/day
Wild-type
IL6 -/-
Δ Food intake (g)
Study day

Fig. 6

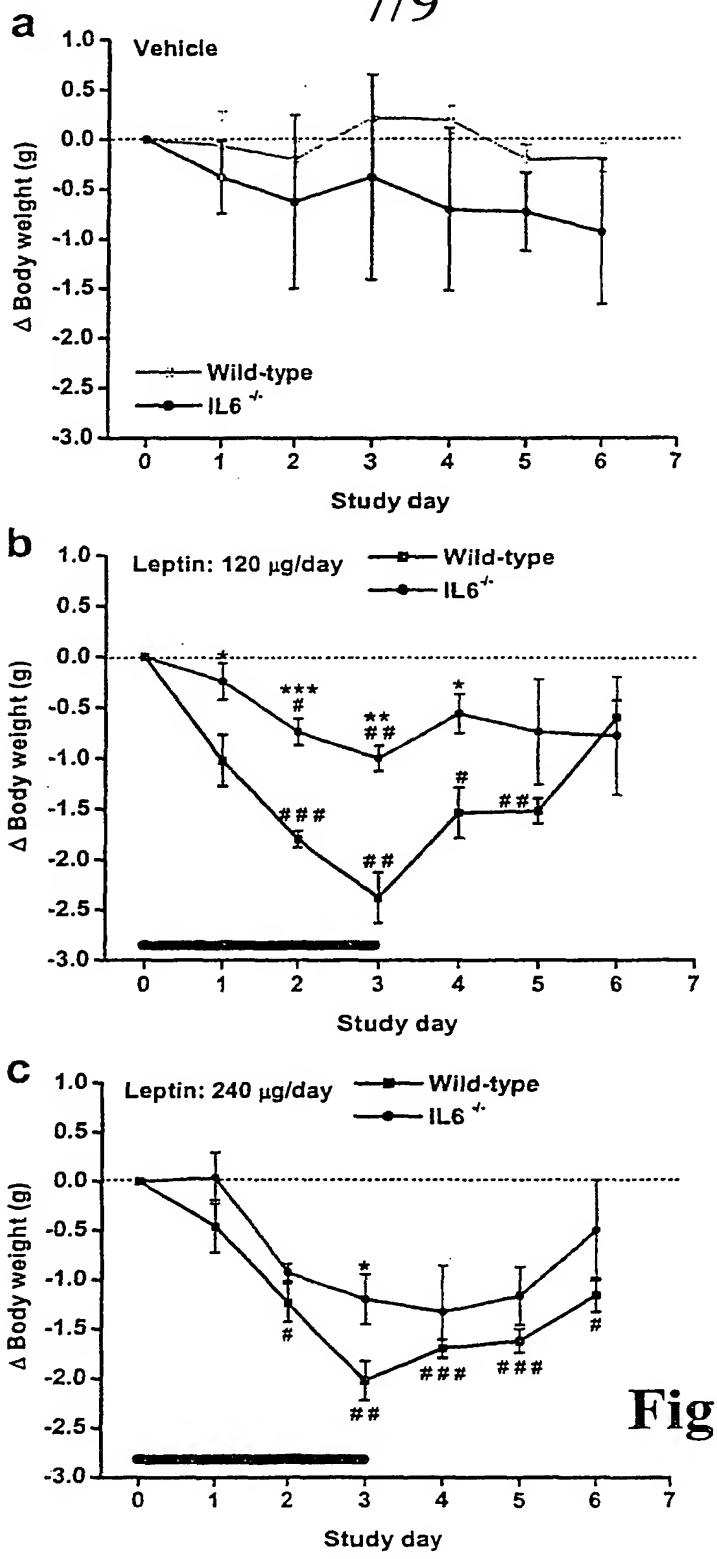
a
Vehicle
Δ Body weight (g)
Wild-type
IL6 -/-
Study day
b
Leptin: 120 µg/day
Wild-type
IL6 -/-
Δ Body weight (g)
Study day
c
Leptin: 240 µg/day
Wild-type
IL6 -/-
Δ Body weight (g)
Study day

Fig. 7

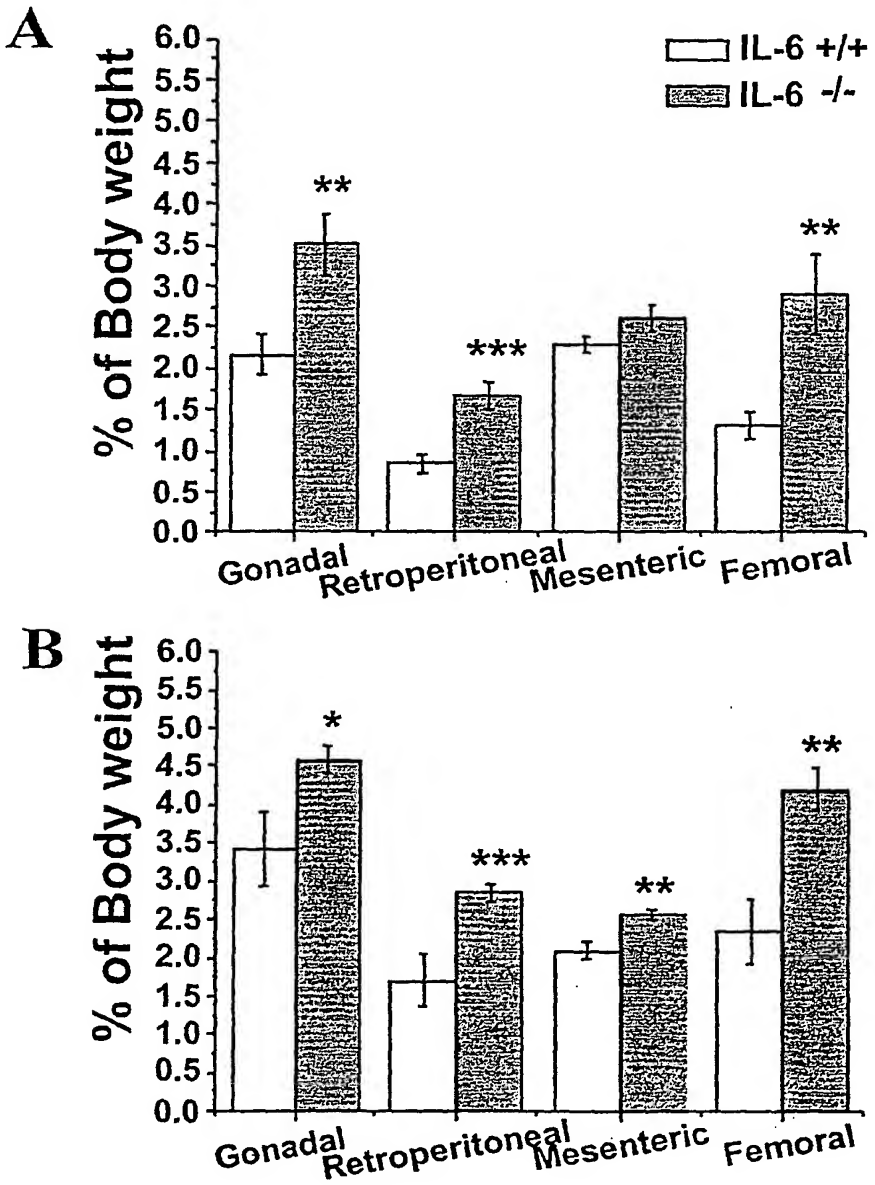
A
% of Body weight
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
IL-6 +/+
IL-6 -/-
**
***
**
Gonadal
Retroperitoneal
Mesenteric
Femoral
B
% of Body weight
*
***
**
**
Gonadal
Retroperitoneal
Mesenteric
Femoral

Fig. 8

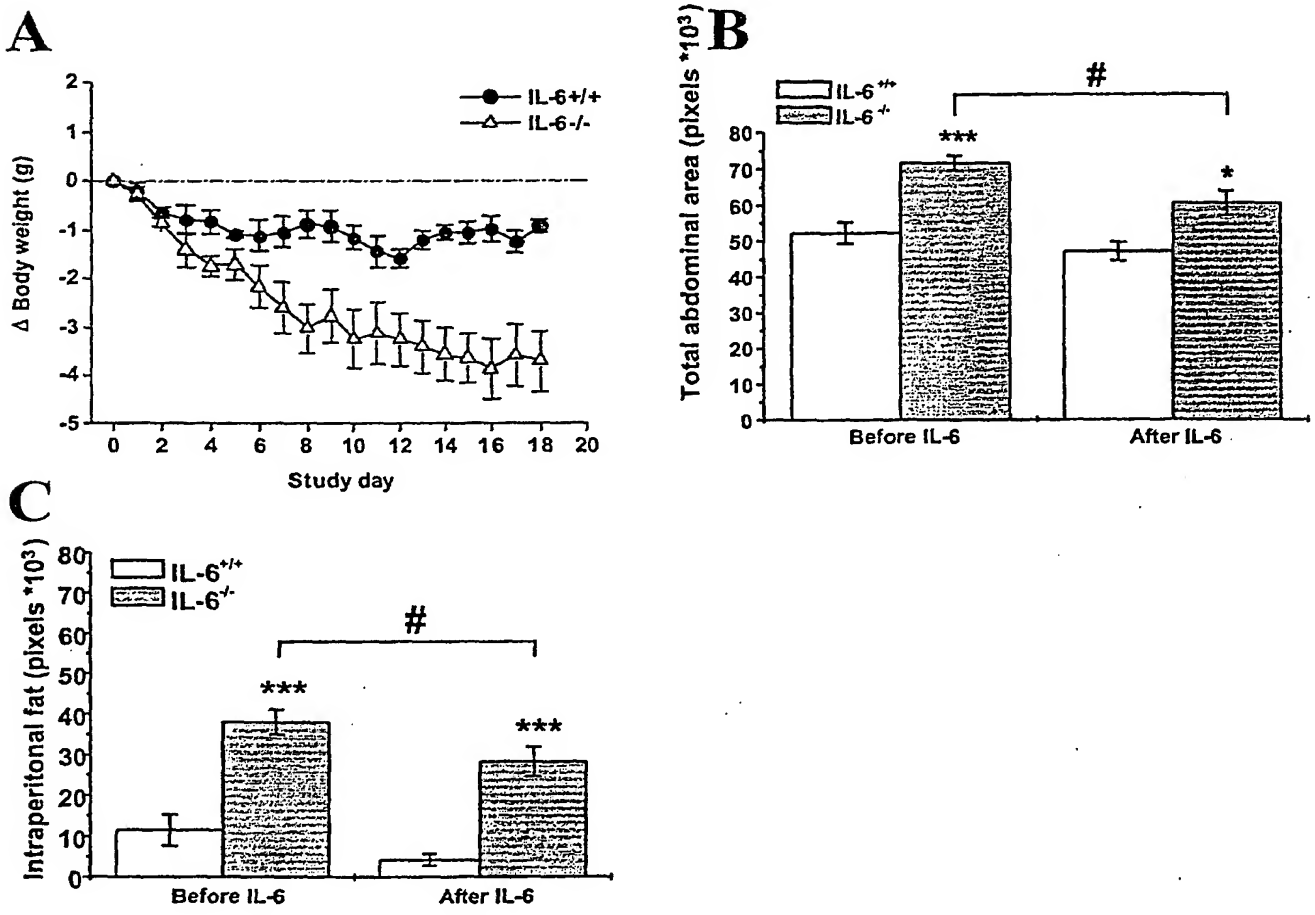
A
Δ Body weight (g)
IL-6+/+
IL-6-/-
Study day
B
Total abdominal area (pixels *10³)
IL-6+/+
IL-6-/-
#
***
*
Before IL-6
After IL-6
C
Intraperitonal fat (pixels *10³)
IL-6+/+
IL-6-/-
#
***
***
Before IL-6
After IL-6

Fig. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01491

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 38/20, A61K 38/22, A61P 3/04, A61P 3/06
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	File WPI, Derwent accession no. 1993-269760, TORAY IND INC: "Hypolipidaemic drug for efficient cholestrerol redn. in blood and compatibility - comprises interleukin 6 prepd. by human cell culture excluding contamination and antibody prodn. on admin. into body, avoiding enzymatic reaction redn."; & JP,A,5186367, 19930727, DW199334	1-13,17-27
Y		14-16,28-30
	--	
X	WO 9732022 A2 (AMGEN INC.), 4 Sept 1997 (04.09.97), see claim 13	1-2,17-18
Y		14-16,28-30
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 November 2000	22 -11- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Palmcrantz/GH Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01491

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NeuroReport, Volume 7, 1996, Carlos R. et al, "Anorexia induced by activators of the signal transducer gp 130" page 841 - page 844	1-13,17-27
X	Am. J. Physiol., Volume 275, 1998, Davide Agnello et al, "Leptin causes body weight loss in the absence of in vivo activities typical for cytokines of the IL-6 family" page 913 - page 919	1-7,9-13, 17-23,25-27
A	DIALOG Information Services, File 155, MEDLINE, DIALOG accession no. 10095030, MEDLINE accession no. 98099248, Loffreda S. et al; "Leptin regulates proinflammatory immune responses"; & FASEB journal (UNITED STATES) Jan 1998, 12 (1) p57-65	14-16,28-30
A	International Immunology, Volume 6, No 12, 1994, Bruno F. DiCosmo et al, "Local production of human IL-6 promotes insulitis but retards the onset of insulin-dependent diabetes mellitys on non-obese diabetic mice" page 1829 - page 1837	11

INTERNATIONAL SEARCH REPORT

[redacted]ational application No.
SE99/01018

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 17-30
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet *

2. ☒ Claims Nos.: 1-2,14,17-18,28
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet **

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE99/01018

box. I,1
*

Claims 17-30 relate to methods of treatment of the human or animal body by therapy (Rule. 39.1.(iv)). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds.

box. I,2
**

The wordings "a substance" and "interleukin-6(IL-6) receptor agonist "in claims 1-2,17-18 and the wording "a factor that will intensify the effect of said interleukin-6(IL-6) receptor agonist" in claims 14 and 28 do not comply with the requirements of clarity and conciseness according to PCT Article 6. The international search has therefore mainly been focused on the examples given in the application i.e. interleukin-6 and leptin.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/11/00

national application No.
PCT/SE 00/01491

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9732022 A2	04/09/97	AU 2317397 A	16/09/97
		CA 2247503 A	04/09/97
		CN 1217023 A	19/05/99
		EP 0912739 A	06/05/99
		IL 125933 D	00/00/00